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ORIGINAL PAPER

Screening *Azadirachta indica* tree for enhancing azadirachtin and oil contents in dry areas of Gujarat, India

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Abstract: *Azadirachta indica* seed samples were collected from four different agro-ecological regions (AERs) viz., AER-2, AER-4, AER-5A and AER-5B of Gujarat state, India during 2000 to 2002 with an aim to assess variability in azadirachtin, oil and fatty acids content of the seeds and assess correlation of these parameters with morphological characters. Azadirachtin, oil and fatty acids content indicated significant (p<0.01) variations between years and AERs in Gujarat. The azadirachtin ranged from 142 to 9 527 μg·g^{-1} seed kernel with an average of 2 426 μg·g^{-1} for the state. AER-5B recorded highest azadirachtin and oil content. Fatty acid composition was found to be affected by environmental factors particularly varying degree of annual rainfall and temperature during fruit ripening period. Conclusively trees growing in AER-5B performed better in term of azadirachtin, oil and stearic acid content. Tree girth at breast height showed no significant relation with these biochemicals.

Keywords: agro-ecological zones; fatty acids; girth-class; neem seed; variability

Introduction

In India, Neem (*Azadirachta indica* A. Juss) has long been recognized for its multifarious properties ranging from pharmaceuticals, pesticidal, religious to biofuel purposes (Gupta and Sharma 1998; Pinzi et al. 2009). However, it gained tremendous importance at the global level after identification of its pesticidal property against locust by Pradhan et al. (1962) and further after characterization of 'azadirachtin' by Zanno et al. (1975) as an active principle present in the Neem seed kernel. Azadirachtin, a tetranortriterpenoid, has been rated as the most potent naturally occurring insecticide (Schroeder and Nakanishi 1987) among all the limonoids found in Neem seed kernel. It is found in different parts of the Neem tree, but it is concentrated in seed kernel of mature fruits (Schmutterer 1981). Various studies have been undertaken on bioefficacy of Neem seed extracts on more than 400 insect pests (Schmutterer and Singh 1995). Studies have also been carried out on the azadirachtin variation in trees growing in different climatic conditions (Ermel et al. 1984; 1986; Rengasamy et al. 1993; Kumar et al. 1995; Bally et al. 1996; Gupta et al. 1998; Kaushik et al. 2007). Variations of Azadirachtin and oil content in different ecotypes and provenances have also been reported (Rengasamy et al. 1993; Gupta et al. 1998; Sidhu et al. 2003; Momchilova et al. 2007; Demirbas 2009). However, till now, very little has been published particularly on azadirachtin variation in seeds growing in different agro climatic zones in India. Moreover, all these studies have been focused on a limited number of samples, and not on the basis of extensive surveys. It is highly essential to understand the geographical variations in seeds, growing in different parts, for identification of region specific plus trees. The knowledge on the range of azadirachtin variation and the possible factors causing this variation has special significance to selection and clonal propagation of high azadirachtin containing planting stock.

A network on 'Integrated Development of Neem' was created by NOVOD Board (National Oilseeds and Vegetable Oils Development Board), Ministry of Agriculture, India in 1999 with an objective of collection, conservation, phenological and chemical evaluation, and mass propagation of selected Neem trees in India. Under this network, Arid Forest Research Institute has collected seeds from different zones of Gujarat and evaluated for their chemical constituents. This paper is a part of the study carried

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out under the network programme to screen large number of neem seed samples for azadirachtin, oil and fatty acids content from different agro-ecological zones of Gujarat with an objective to understand the influence of girth class, and zone's annual rainfall, mean maximum, mean minimum temperature of June (the fruit ripening period in Gujarat) on these secondary metabolites.

Material and methods

Study area

This study was carried out in Gujarat state situated on the west coast of India and boasts of a 1 600 km long coastline of the Arabian Sea in the western and south western frontiers of the state. It is situated between 20°1' and 24°7' N latitudes and 68°4' and 74°4' E longitudes. Gujarat stretches from Kutch in the West to Daman in the South. In the East, lies the hilly region of the Aravallis. It even shares an international border with Sindh province of Pakistan, which lies on the northwest side of the state. Gujarat is divided into five agro-ecological zones (AER-2, 4, 5, 6, and 19) as shown in map of Gujarat (Fig. 1). Since zone AER-5 is a large area, it was further divided into two zones (AER-5A and AER-5B) on the basis of different climatic conditions. Brief characteristics of these four AERs zones (Seed collection zones i.e. AER 2, 4, 5A & 5B) along with five years (1998–2002) annual rainfall (mm), mean maximum and mean minimum air temperature (°C) are given in Table 1. Average rainfall of agro-ecological zones for five years (i.e., 1998 to 2002) during the seed collection time were 367 mm, 586 mm, 618 mm and 760 mm, respectively in AER-2, AER-4, AER-5A and AER-5B. The rainfall in 1998, 1999, 2000, 2001 and 2002 were 821, 427 457, 645, and 506 mm, respectively. Mean maximum temperature of June (average of five years i.e., 1998 to 2002) was the highest in AER-4 and the lowest was in AER-5B, whereas mean monthly minimum temperature was the highest in AER-2 and the lowest was in AER-5B agro-ecological zone (Table 1). Soil of the study area varied widely from aridisol to deep coastal alluvium (Table 1).

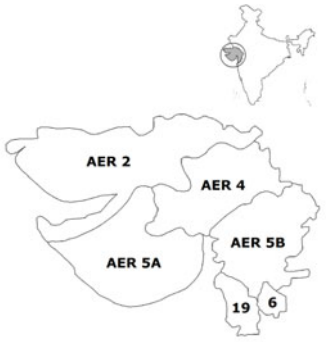

Fig. 1 Agro-ecological zones of Gujarat state in India.

Table 1. Agro-ecological zones, rainfall, maximum and minimum air temperature of June and climatic characteristics of different Agro-eco regions (AERs) of Gujarat state, India

Agro-eco region	Year	Rainfall (mm)	Air Temp. (°C)		Climatic & soil characteristics	Areas and districts
			Max.	Min.		
AER- 2	1998	484	43.6	24.4	Hot arid ecoregion with desert and saline soils	Banaskantha (Palanpur), Bhuj, Rann of Kachchh, western parts of Mehsana, north-western parts of Surendranagar, northern parts of Rajkot and Jamnagar districts.
	1999	326	38.3	25		
	2000	303	44.8	26.3		
	2001	451	34.7	26.5		
	2002	171	36.9	27.3		
AER- 4	1998	735	43.6	28	Hot semi-arid ecoregion with alluvium-derived Soils	Gujarat plains, including the districts of Ahmedabad, Sabarkantha, (Himatnagar), Mehsana and Surendranagar (eastern parts), and northern parts of Kheda.
	1999	579	40.3	23		
	2000	560	42.6	23.9		
	2001	735	35.5	26.1		
	2002	322	38.8	27.8		
AER- 5A	1998	775	42.7	24	Hot semi-arid ecoregion with medium, deep black soils	Amreli, Bhavnagar, Junagadh, Panchmahal (Godhra), fringe of Ahmedabad district.
	1999	428	39.9	24.6		
	2000	444	40.3	25.1		
	2001	750	34.1	26.7		
	2002	685	35.7	24.5		
AER-5B	1998	1292	41.5	24	Hot semi-arid ecoregion with medium, deep black soils, deep black coastal alluvium, sub-humid	Southern half of Kheda, Vadodara, Bharuch, Rajkot (southern parts), nd Surat (north-eastern parts), and southern
	1999	375	37.6	23.1		
	2000	520	38.4	21.3		
	2001	-	33.3	26.3		
	2002	854	35.1	26.8		

Survey, fruits collection and processing

Survey was done in collaboration with Gujarat Forest Department for selection and identification of candidate trees on the basis of morphological characters of Neem trees growing in different agroclimatic zones of Gujarat. Survey was conducted in all

the five AER zones of the state. Good plantations were available only in the AER-2, AER-4, AER-5A and AER-5B from which a total number of 367 trees were selected. Since it was difficult to know the actual age of the trees, their GBH (girth at breast height) were measured to get an idea about the variation due to age of a tree. Seed samples were collected in the last week of June for three consecutive years 2000, 2001, and 2002 from the selected individual candidate trees, growing in the four different agroclimatic zones. A total of 367 seed samples from the selected trees belonging to AER-2, AER-4, AER-5A and AER-5B were collected for assessment of azadirachtin, oil and different fatty acids content in their seed kernel. Fully ripen yellow fruits were collected directly from the branches of individual trees. Fruits were depulped manually by hand, and washed thoroughly with clean water to remove the traces of pulp from the seed coat. The depulped and washed seeds were dried in shade before packing them in cotton bags. Seed samples of individual trees were packed with identity tag in muslin bags.

Equipment and material

Azadirachtin estimation was performed using a Waters LC Module I Quaternary Automated Liquid Chromatograph equipped with autoinjector, high-sensitivity tunable UV and photodiode array detectors, and Novapak RP-18 column (3.9 mm × 150 mm). The chromatograms and data were acquired and processed with the Waters Millennium 2010 Chromatography Manager version 2.1 software. The photodiode array spectrum was recorded on a Waters 996 Photodiode Array Detector. HPLC grade acetonitrile was procured from Merck (India). Ethanol was obtained by distilling spirit. Azadirachtin standard (96%) was procured from Trifolio-M (Germany). The samples were prepared in Borosil screw-capped centrifuge tubes (15 mL). A thermostatic serological water bath was used for heating the samples. A REMI Revolutionary Research Centrifuge (Model R-23), which could accommodate 36 tubes, was used for centrifugation of the samples. Samples were filtered through Swinnex polypropylene 25-mm filter holders (Millipore, USA) containing Durapore 0.22 μm filters. Azadirachtin samples were filtered into Waters autosampler vials (4 mL). The mobile phase for HPLC was filtered through a Millipore sample clarification kit fitted with Durapore 0.45 μm, 47 mm filters (Millipore, USA).

Azadirachtin, oil and fatty acids estimation

Azadiracthin, total oil and fatty acids (oleic, palmitic, stearic and linoleic acids) content of the neem seeds was determined as per the method standardized in TERI's laboratory (Kaushik 2002).

Statistical analysis

All the data were analyzed using SPSS statistical package 'version 2000'. Tree girth was analyzed using one-way ANOVA considering agro-climatic zone as fixed factor and tree girth as dependent variable. Data on azadirachtin, oil and fatty acids were analyzed using two-way ANOVA. In this data of three successive years and agro-climatic zone were factors and different data were dependent variables. Data was clustered into different groups on basis of year of collection, agro–ecological zone, and tree girth for analyzing these results statistically. Duncan Multiple Range Test (DMRT) was performed at 5% significance level to observe the homogeneous sub-set between the years as well as the agro-climatic zones. A Pearson correlation coefficient was observed to find out relationship between different variables of seeds, rainfall, air temperature (mean maximum and minimum of June) and tree girth. Regression equations were also observed to find out relation between azadirachtin content regressed against the GBH, oil content, fatty acids and rainfall in different years, whereas palmitic acid was regressed against GBH and other variables, but only significant results are presented.

Results

Tree growth variables

Girth at breast height (GBH) of *A. indica* tree did not vary (p>0.05) among years as well as AERs. Average GBH for the state was 128.1 cm. However, tree GBH was relatively greater (p<0.05) in 2002 than in 2000 (indicated by DMRT). Among the AERs, tree GBH was highest in AER-4, whereas the lowest GBH was recorded in AER-2, though the variation was not-significant (p>0.05). However, year × AER interaction was significant (p<0.01) and the GBH was highest in 2002 in AER-4, while the lowest GBH was in AER-2 in 2000 (Table 2).

Table 2. Tree girth at breast height and azadirachtin content influenced by year and agro-eco region. Values in 3rd and 4th column are mean ±SE.

Agro-eco region (AER)	Year	Girth at breast height (cm)	Azadirachtin ($\mu g \cdot g^{-1}$ seed kernel)
AER- 2	2000	111.69±4.87	1752.43±87.60
	2001	135.20±6.81	2852.88±248.83
	2002	130.55±7.70	3634.18±314.96
AER- 4	2000	118.85±5.76	924.88±68.12
	2001	139.45±4.85	2011.14±238.71
	2002	162.05±10.15	3243.41±354.10
AER- 5A	2000	136.14±10.02	2169.25±124.90
	2001	125.37±7.05	2900.61±260.89
	2002	121.72±7.84	2515.25±310.03
AER- 5B	2000	133.58±15.03	2524.31±255.72
	2001	-	-
	2002	145.00±8.65	5541.00±921.75
Two-way ANOVA			
F value	d.f.		
Y	2	2.07	35.84
Z	3	1.84	10.43
Y × Z	5	2.14	5.33
P value			
Y	2	NS	<0.001
Z	3	NS	<0.001
Y × Z	5	<0.01	<0.001

Abbreviations: Y- Year and Z - Agroclimatic Zone

Azadirachtin content and variations

A large variation in azadirachtin level was recorded in 367 seed samples. The azadirachtin content ranged from 142 to 9 527 μg·g^{-1} seed kernel and an overall average of the whole population was 2 426 μg·g^{-1}. In order to view the frequency distribution of the trees based on azadirachtin content, they were clustered into ten different classes having an interval of 1000 μg·g^{-1}, ranging from 0–1 000 μg·g^{-1} to 9 000–10 000 μg·g^{-1}. There was a wide variation in the number of tress distributed in different azadirachtin classes as recorded in 2000, 2001, and 2002 (Fig. 1). Majority of trees fall within a range of 1 000–3 000 μg·g^{-1} level, irrespective of the year of collection (Fig. 2). Sixteen trees showed higher azadirachtin content (above 6 000 μg·g^{-1} seed kernel), which is far above the average azadirachtin content (2 426 μg·g^{-1}) recorded for the state. Two-way ANOVA showed wide variation in Azadirachtin content both within the years and the AERs (Table 2). Irrespective of AERs, azadirachtin increased significantly (p<0.01) from 1 792.5 in 2000 to 3 115.2 μg·g^{-1} in 2002. The increase in azadirachtin content was 1.5-fold in 2001 and 1.74-fold in 2002, when compared with that in 2000. While considering agro-eco regions, the average azadirachtin level of four agro-eco regions was significantly (p<0.01) different. Maximum average azadirachtin content (3 347 μg·g^{-1}) was recorded in zone AER-5B and minimum (2 037 μg·g^{-1}) was in AER-4. But DMRT indicated insignificant (p>0.05) difference in azadirachtin content among AER-2, AER-4 and AER-5A (Table 2). Year × AER interaction term indicated the highest (p<0.01) azadirachtin content in AER-5B in 2002 and the lowest in AER-2 in 2000.

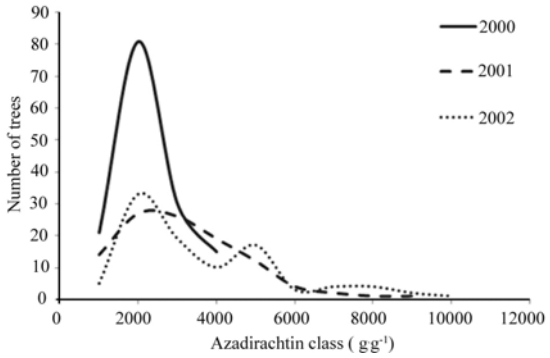

Fig. 2 Frequency curve of Neem tree on the basis of azadirachtin content recorded in three different year climatic zone of Gujarat, India.

Oil and fatty acid content

Oil content of Neem seed kernel ranged from 41.33% to 51.03%. DMRT indicated an increase in oil content significantly (p<0.05) from 42.41% in 2000 to 46.65% in 2002 (Table 3). Among the AERs, average oil content was the highest (p<0.01) in AER-5B and the lowest was in AER-2. However, DMRT indicated a non-significant (p>0.05) difference in seed oil in AER-2 and AER-4. Significant (p<0.01) year × AER interaction term indicated the highest oil content in AER-5B in 2002 and the lowest oil content was in AER-4 in 2000.

Table 3. Average seed oil content and fatty acid concentration (% of oil) in seed kernel of *A. indica* influenced by year and agroclimatic zone.

Agro-eco region (AER)	Year	Oil (%)	Fatty acids (%)			
			Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
AER- 2	2000	42.28±0.44	21.00±0.33	11.03±0.23	53.92±0.35	13.49±0.33
	2001	43.89±0.60	20.53±0.35	7.69±0.21	50.63±0.51	19.30±0.49
	2002	44.67±0.67	18.97±0.32	17.54±0.57	48.96±1.03	14.48±0.34
AER- 4	2000	41.33±0.67	20.43±0.54	10.90±0.42	46.78±0.44	15.56±0.40
	2001	42.33±0.95	17.16±0.33	7.13±0.23	53.26±0.42	19.85±0.31
	2002	46.31±0.75	17.00±0.32	15.47±0.38	54.86±0.51	14.48±0.35
AER- 5A	2000	43.52±0.47	21.12±0.40	12.44±0.33	50.47±0.51	15.63±0.48
	2001	46.98±0.43	18.72±0.22	6.67±0.20	47.51±0.56	24.28±0.65
	2002	47.16±0.43	19.21±0.15	17.18±0.23	48.85±0.49	14.49±0.32
AER- 5B	2000	42.17±0.68	20.75±0.88	12.12±0.55	49.35±0.90	17.50±0.86
	2001	-	-	-	-	-
	2002	51.03±1.79	17.59±0.26	14.56±0.79	54.43±0.73	13.42±0.66
Two-way ANOVA						
F value	d.f.					
Y	2	41.53	34.30	585.85	4.88	188.10
Z	3	14.58	12.47	5.58	14.96	16.51
Y x Z	5	4.61	4.30	8.47	28.29	8.75
P value						
Y	2	<0.001	<0.001	<0.001	<0.01	<0.001
Z	3	<0.001	<0.001	<0.01	<0.001	<0.001
Y x Z	5	<0.001	<0.01	<0.001	<0.001	<0.001

Abbreviations: Y- Year and Z - Agroclimatic Zone

The highest contribution of fatty acids in the total oil content was for oleic acid i.e., 50.75%. The contribution of other fatty acids namely palmitic acid, stearic acid and linoleic acid were 19.67%, 11.61% and 16.66%, respectively. These all fatty acids significantly varied ($p < 0.01$) due to year of collection and AERs (Table 3). Considering an average of the AERs for the year, palmitic acid and oleic acid were the highest ($p < 0.01$) in 2000, linoleic in 2001 and stearic acid in 2003. The lowest contributions of stearic and oleic acids were in 2001 and those of palmitic acid and linoleic acids were in 2002. Considering AERs, palmitic and oleic acids were the highest ($p < 0.01$) in AER-2. But the contribution of stearic and linoleic acids were the highest ($p<0.05$) in AER-5B and AER-5A, respectively. The lowest contribution of palmitic and oleic acids were in AER-4 and AER-5A, respectively, whereas stearic and linoleic acids showed their lowest value in AER-2. Significant ($p < 0.01$) year × AER interaction terms showed the highest value of palmitic acids in ARE-2 in 2000, stearic acid in AER-2 in 2002, oleic acid in AER-4 in 2002 and linoleic acid in AER-5A in 2001 (Table 3).

Correlations and regressions

Tree girth at breast height (GBH) did not show relations with either rainfall in different years or oil and azadirachtin content (Table 4). But the girth was negatively related with mean maximum temperature of June (r = -0.118, $p<0.01$). A weak positive correlation of azadirachtin content with oil (r=0.253, $p<0.01$, n=341) and rainfall of previous (Y-1) year (r = 0.132, $p<0.05$) was observed in present studies. Palmitic acid (2000, 2001 and 2002) showed a negative relation with oil, azadirachtin, mean maximum air temperature, rainfall in the same year (Y) i.e., 2000, 2001 and 2002 and the previous (Y-1) i.e., 1999, 2000 and 2001, respectively, but showed a positive relation with combined rainfall of the last two year [(Y-1)+(Y-2)] i.e., 1999+1998, 2000+1999 and 2001+2000 and mean minimum air temperature of June same year. Stearic acid was positively related with oil, azadirachtin, mean minimum air temperature of June of the same year and rainfall of one year before (Y-1), but negatively related with rainfall in the same year. Oleic acid was positively related, whereas linoleic acid was negatively related with both mean maximum and mean minimum temperature of June. However, azadirachtin content showed negative relation (r = -0.308, $p < 0.01$) with mean maximum and positive relation (r = 0.219, $p < 0.01$) with mean minimum temperature of June. Regression equation to estimate azadirachtin content showed inverse relation (R^2=0.027, $p < 0.01$, n=341) with palmitic acid content and sigmoidal (R^2=0.011, $p < 0.05$) relation and linear (R^2=0.017, p=0.0139) relation with rainfall in the same year and previous year (Y-1), respectively (Fig. 3). However, palmitic acid was inversely related (R^2=0.016, p=0.0216) with GBH.

Table 4. Correlation coefficient (r) between different variables of *Azadirachta indica* in Gujarat state, India

Variable	GBH	Palmitic acid	Strearic acid	Oleic acid	Linoleic acid	Oil	Aza
Oil	NS	-0.247**	0.150**	-0.203**	NS	-	0.253**
Aza	NS	-0.161**	0.120*	NS	NS	0.253**	-
Rainfall (Y)	NS	-0.149**	-0.131**	0.136**	0.208**	0.156*	NS
RF (Y-1)	NS	-0.208	0.331	NS	-0.176**	0.205**	0.132*
RF (Y-2)	NS	NS	NS	NS	NS	NS	NS
RF (Y-1)+(Y-2)	NS	-0.159**	NS	NS	NS	NS	NS
Temp.(max.)	-0.118	0.312**	NS	0.342**	-0.462**	-0.402**	-0.308**
Temp(min.)	NS	-0.260**	0.221**	0.114*	NS	0.344**	0.219**

Significant at *$p < 0.05$; **$p < 0.01$; NS, not-significant ($p > 0.05$)

Discussion

Tree girth and azadirachtin content

Girth of *A. indica* trees varied widely but variation was not significant ($p>0.05$) between the years and AERs. However, the variations in azadirachtin was significant ($p<0.01$). Azadirachtin variation between individual trees and between different ecotypes has been studied by many scientists (Rengasamy et al. 1993; Kumar et al 1995; Gupta et al. 1998; Kaushik et al. 2007). Ermel et al. (1986) observed that individual trees growing in the same environment exhibit significant difference in their azadirachtin level. They also found that the highest azadirachtin was not restricted to a specific ecotype but it was from a single tree of different origins. This was in contrast to the earlier report of Schmutterer and Zebitz (1984), where they found marked differences in yield of azadirachtin in seeds collected from different sources. Our investigation also exhibits that individual genotype exhibits large variation. However, these individual genotypic variations were observed in small fraction of the sample. It is clearly demonstrated that agro–climatic conditions played an important role in azadirachtin synthesis. AER-5B zone seems to the best area for growing plants for azadirachtin extraction in Gujarat and was probably due to greater rainfall received in this region as compared to the other AERs and the lowest temperature difference between mean minimum and mean maximum of June. A linear increase in azadirachtin content (R^2= 0.017, $p<0.05$, n = 347) with rainfall in the previous year supported this inference (Fig. 3). But it was worth mentioning that increase in azadirachtin content from 2000 to 2002 particularly in year 2002, which was relatively a drought year, was probably due to water stress period during fruiting probably facilitate azadirachtin biosynthesis, but for this tree utilized stored soil water of the previous year. Shridharan et al. (1998) observed a negative correlation

between azadirachtin content and the total number of rainy days during the fruiting season in Tamil Nadu, India. In present study we did not find relation between azadirachtin content and rainfall of the same year, but a positive relation (r=0.132, p<0.05) with rainfall in previous year. It is because fruits are ripen in June and main rainy period is from July to August in Gujarat. Thus rainfall of same year can not influence any biochemical synthesis in ripen Neem fruits. Lowest Azadirachtin content in AER-4 and AER-2 were due to aridisol and hot arid/ semiarid climatic conditions resulting in the highest differences in mean maximum and mean minimum temperature of June. It has also been shown by a negative (r=-0.308, p<0.01) and positive (r=0.219, p<0.01) relations of mean maximum and mean minimum temperature of June, the time of fruit ripening. Elteraifi and Hassanali (2010) observed that neem trees growing in regions with moderate climate, average rainfall of 400 mm, and altitude of more 470 m above sea level in Sudan, proved to be rich in azadirachtin content. Whereas, trees growing in lower altitudes, alluvial or sandy soil, with hot climate reflected very low azadirachtin content (Elteraifi and Hassanali 2010). Kaushik et al (2007) also recorded the lowest azadirachtin content of the tree seeds collected from desert areas of north-western India.

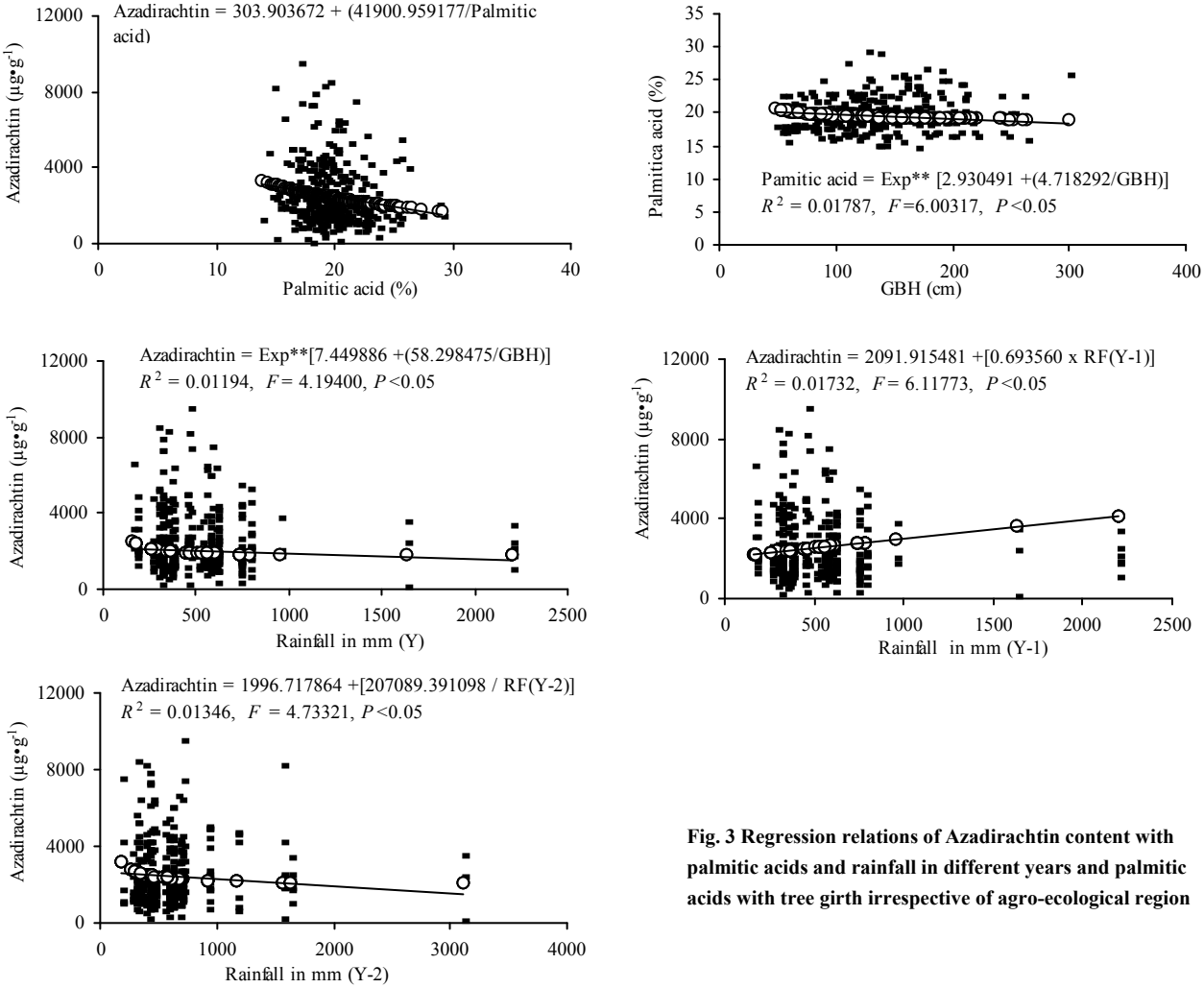

Fig. 3 Regression relations of Azadirachtin content with palmitic acids and rainfall in different years and palmitic acids with tree girth irrespective of agro-ecological region

Significant variations in azadirachtin content between years is in consonance with the observation of Sidhu and Behl (1996) and Bally et al. (1996), who have reported a seasonal variation and annual azadirachtin fluctuations. The reasons of fluctuation in azadirachtin content are likely to be due to climatic and nutritional factors. A non-significant relation between tree GBH and azadirachtin content indicate that azadirachtin levels were not greatly influenced by the tree age (Table 4). However, highest level of azadirachtin in girth class of 145.00 cm in AER-5B as compared to that in the girth class of 162.05 cm in AER-4 indicated that highest azadirachtin can be extracted from middle girth class trees with relatively better rainfall areas (Table 2).

Oil and fatty acids content

Oil content followed similar pattern to that of azadirachtin content indicated by an increase in oil concentration from 2000 to 2002, similar to the quantity of rainfall received in the previous years (i.e., 401.1 mm, 424.6 mm and 720.1 mm in 1999, 2000, and 2001, respectively). This suggests that *A. indica* tree utilized

the water stored in the soil profile from the previous rainfall for biosynthesis of oil. Highest oil content ($p < 0.01$) in AER-5B and lowest in AER-2 supports that water stress (relatively low rainfall) and greater differences in mean maximum and mean minimum temperature in June in latter region affected oil biosynthesis. Significant positive relation between oil content and the number of sunshine hours from September to March in Tamil Nadu areas of India suggested the effect of sunshine (light intensity) in azadirachtin biosynthesis (Shridharan et al. 1998). However, variations in oil content due to soil characteristics might also play an important role in growth and productivity of biochemicals. Despite variations in rainfall, a non-significant ($p>0.05$) difference in seed oil in AER-2 and AER-4 was probably due to sandy nature of the soil in these both regions that heated in day time and cooled in night making a greater difference between mean minimum and mean maximum temperature (i.e., June in this study). Thus the highest oil content ($p<0.01$) in AER-5B in 2002 was contributory effects of greater rainfall in 2001 (previous year) and relatively drought period and water stress during oil biosynthesis period. Lowest temperature (maximum, minimum and their difference) of this zone might have also influenced the biosynthesis. Azadirachtin and oil content from 12 different locations of Tamil Nadu in India also showed wide variations, but showed some correlations with climatic factors (Shridharan et al. 1998).

Among the fatty acids, oleic acids contributed to about 51% of the total oil, whereas palmitic, stearic and linoleic acids contributed 19.67%, 11.61% and 16.66%, respectively. Muñoz-Valenzuela et al. (2007) also observed similar pattern of different fatty acids i.e., oleic acid 45.6%, linoleic acid 16.8%, palmitic acid 17.21%, stearic acid 15.2%, and linolenic acid 1.3% in neem seed kernel in Yaqui Valley in Southern Sonora, México. In their study Muñoz-Valenzuela et al. (2007) also found insignificant relation between tree age and oil yield as observed in present study.

Wide ($p < 0.01$) variations in all the fatty acids between the years and the agro-eco regions suggested the effect of climatic factors on their biosynthesis. The highest ($p < 0.01$) concentration of palmitic acid and oleic acid in 2000 was probably because of relatively less rainfall as compared to the other years. It was evinced by a decrease in concentration of palmitic acid from 2000 to 2002 and indicated by a negative relation of palmitic acid with rainfall in same year ($r = –0.149$, $p<0.01$, n=340) and in previous year ($r= –0.208$, $p < 0.01$). However, the highest concentration of linoleic in 2001 suggested a positive response of this fatty acid with rainfall in same year ($r=0.208$, $p<0.01$) but a negative relation of this fatty acid with oleic acid ($r = –0.311$, $p < 0.01$) suggested possible conversation of latter into linoleic acid or relatively greater synthesis of linoleic acid under relatively greater rainfall period. But probably saturation/ conversion of these fatty acids into stearic acid resulted in an increased concentration of stearic acid in 2003. This was also suggested by highest ($p<0.01$) concentrations of palmitic and oleic acids in AER-2, which is a hot arid region, where quantity of rainfall was less resulting in water stress situation. Elteraifi and Hassanali (2010) observed rainfall as the major factor affecting the level of azadirachtin and oil and the optimal rainfall was 717 mm. Thus highest concentration of stearic and linoleic acids were related with rainfall which was highest in AER-5. But relation of palmitic and linoleic acids were significantly ($p < 0.01$) negative and positive, respectively with rainfall (year × AER interaction terms) as indicated by highest value of palmitic acids in ARE-2 in 2000 and that of linoleic acid in AER-5 in 2001.

Conclusions and recommendations

This study indicated that *Azadirachta indica* trees growing in different habitats in the Gujarat showed wide variations in azadirachtin, oil and fatty acids content. The azadirachtin ranged from 142 to 9 527 $\mu g \cdot g^{-1}$ with an average of 2 426 $\mu g \cdot g^{-1}$ for the state. Azadirachtin and fatty acids content increased from 2000 to 2002 and the increase was 1.74-fold for azadirachtin. Both azadirachtin and oil were the highest in AER-5B and lowest in AER-4/AER-2 and was influenced by mean air temperature of June. Oleic acids contributed about 51% of the total oil, whereas palmitic, stearic and linoleic acids contributed 19.67%, 11.61% and 16.66%, respectively. Palmitic and oleic acid were highest in 2000, linoleic in 2001 and stearic acid in 2003 indicating the contributory effects of rainfall, air temperature and water stress i.e., environmental factors. Conclusively trees growing in AER-5B performed better in term of azadirachtin, oil and stearic acid content. However, concentrations of palmitic and oleic acids were relatively greater in AER-2. This study gave us idea about genotype and environmental effect on azadirachtin and lipid synthesis. Thus future investigation should be focused on clonally propagated trees of AER-5B to increase the production of these valuable bioproducts.

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References

Bally ISE, Ruddle L, Simpson B. 1996. Azadirachtin levels in Neem seed grown in Northern Australia. In: International Neem Conference Feb 4-9, Queensland, Australia, p.17.

Demirbas A. 2009. Potential resources of non-edible oils for biodiesel. *Energy Sources: Econ Planning Policy*, **4**: 310–314.

Elteraifi IE, Hassanali A. 2010. Oil and azadirachtin contents of neem (*Azadirachta indica* A. Juss) seed kernels collected from trees growing in different habitats in sudan. http://wgbis.ces.iisc.ernet.in/energy/lake2006/programme/programme/lake2006_Pdf/ Intisar.pdf dated 20th January 2010.

Ermel K, Pahlich E, Schmutterer H. 1984. Comparison of the azadirachtin content of neem seeds from ecotypes of Asian and African origin. In: Schnutterer H, Aschier KRS. (eds.) Proceeding of 2nd Int Neem Conf, GTZ, Germany, pp. 91–94.

Ermel K, Pahlich E, Schmutterer H. 1986. Azadirachtin content of neem kernels from ecotypes different geographical locations and it dependence on temperature, relative humidity and light. In: Schnutterer H, Aschier KRS. (eds.), Proceeding of 2nd Int Neem Conf, GTZ, Germany, pp. 171–184.

Gupta PK, Tripathi YC, Prabhu VV, Pal RS. 1998. Variation in fatty oil and azadirachtin contents of neem seed kernels of different geographical origins. In: Gupta BN, Sharma KK. (eds.), *Neem A Wonder Tree*. Dehra Dun, India: ICFRE, pp. 142–148.

Kaushik N. 2002. Determination of azadirachtin and fatty acid methyl esters of *Azadirachta indica* seeds by HPLC and GLC. *Anal Bioanal Chem*, **374**: 1199–1204.

Kaushik N, Singh BG, Tomar UK, Naik SN, Vir S, Bisla SS, Sharma KK, Banerjee SK, Thakkar P. 2007. Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica* A. Jusieu). *Current Science*, **92**: 1400–1406.

Kumar MG, Kumar RJ, Regupathy A, Rajasekaran B. 1995. Liquid chromatographic determination and monitoring of Azadirachtin in Neem ecotypes. *Neem Update*, **1**: 4–5.

Momchilova S, Antonova D, Marekov I, Kuleva L, Damyanova BN, Jham G. 2007. Fatty acids, triacylglycerols, and sterols in Neem oil (*Azadirachta Indica* A. Juss) as determined by a combination of chromatographic and spectral techniques. *J Liquid Chromatography & Related Technology*, **30**: 11–25.

Muñoz-Valenzuela S, Ibarra-López AA, Rubio-Silva LM, Valdez-Dávila H, Borboa-Flores J. 2007. Neem tree morphology and oil Content. In: Janick, J, hipkey A. (eds.), *Issues in new crops and new uses*. Alexandria, VA: ASHS Press, pp. 126–128.

Pinzi S, Garcia IL, Lopez-Gimenez FJ, Luque de Castro MD, Dorado G, Dorado MP. 2009. The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. *Energy Fuels*, **23**: 2325–2341.

Pradhan S, Jotwani MG, Rai BK 1962. The neem seed deterrent to locusts. *Indian Farming*, **12**: 7–11.

Rengasamy S, Kaushik N, Kumar J, Kaul O, Parmar BS. 1993. Azadircahtin content and bioactivity of Neem ecotypes of India. In: Singh RP. (ed.), *Proceedings of World Neem Conference*. New Delhi: IHCO, pp. 207–211

Schmutterer H. 1981. Ten years of Neem research in Federal Republic of Germany. In: Schnutterer H, Aschier KRS, Rembold H. (eds.), Natural pesicide from Neem tree. Proceeding of 1st Int. Neem Conf, GTZ, Germany, pp. 21–32.

Schmutterer H, Singh RP. 1995 List of insect pests susceptible to neem products. In: Schnutterer H. (ed.), The Neem Tree. VCH Verlagsgesellsschaft, Weinheim, Federal Republic of Germany, pp. 326–365.

Schmutterer H, Zebitz CPW. 1984 Effect of methanolic extracts from seed of single neem trees of Africa and Asian origin on the Epilachnan varivestis & Aedes aegypti. In: Schnutterer H, Aschier KRS. (eds.), Natural pesicide from Neem tree. Proceeding of 2nd Int. Neem Confrence, Rottach-Egern, GTZ, Germany. pp. 83–90.

Schroeder N. 1987. A simplified isolation procedure for Azadirachtin. *J Natural Products*, **50**: 241–244.

Sidhu OP, Behl HM. 1996. Seasonal variation in azadirchtins in seeds of Azadirachta indica. *Current Science*, **70**: 1084–1086.

Sidhu OP, Kumar V, Behl HM. 2003. Variability in Neem (*Azadirchta indica*) with respect to azadirchtin content. *J Agri Food Chem*, **51**: 910–915.

Sridharan S, Venugopal MS, Dhaliwal GS, Arora R, Randhawa NS, Dhawan AK. 1998. Effect of environmental conditions on the yield of azadirachtin and oil in neem. In: Ecological agriculture and sustainable Development: Volume 1, Indian Ecological Society and the Centre for research in Rural and industrial development. Chandigarh, India, pp. 510–518.

Zanno PR, Miara E, Nakanaishi K, Elder DL. 1975. Structure of the insect phagorepellant Azadirachtin, application of PRFT/CWD carbon 13 nuclear magnetic resonance. *J Am Chem Soc*, **97**: 1975–1977.